EXHIBIT A

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ORIGINAL REPORT

Phase I Clinical Study of Pertuzumab, a Novel HER Dimerization Inhibitor, in Patients With Advanced Cancer

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Terms in blue are defined in the glossary, found at the end of this issue and online at www.jco.org.

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ABSTRACT

Purpose
Pertuzumab, a recombinant humanized monoclonal antibody (2C4), binds to extracellular domain II of the HER-2 receptor and blocks its ability to dimerize with other HER receptors. Pertuzumab represents a new class of targeted therapeutics known as HER dimerization inhibitors. A clinical study was conducted to investigate safety and pharmacokinetics of pertuzumab and to perform a preliminary assessment of HER dimerization inhibition as a treatment strategy.

Patients and Methods
Patients with incurable, locally advanced, recurrent or metastatic solid tumors that had progressed during or after standard therapy were recruited to a dose-escalation study of pertuzumab (0.5 to 15 mg/kg) given intravenously every 3 weeks.

Results
Twenty-one patients received pertuzumab and 19 completed at least two cycles. Pertuzumab was well tolerated. Overall, 365 adverse events were reported and 122 considered to be possibly drug related. Of these, 116 were of grade 1 to 2 intensity. The pharmacokinetics of pertuzumab were similar to other humanized immunoglobulin G antibodies, supporting a 3-week dosing regimen. Trough plasma concentrations were in excess of target concentrations at doses greater than 5 mg/kg. Two patients, one with ovarian cancer (5.0 mg/kg) and one with pancreatic islet cell carcinoma (15.0 mg/kg), achieved a partial response. Responses were documented by Response Evaluation Criteria in Solid Tumors after 1.5 and 6 months of pertuzumab therapy, and lasted for 11 and 10 months, respectively. Stable disease lasting for more than 2.5 months (range, 2.6 to 5.5 months) was observed in six patients.

Conclusion
These results demonstrate that pertuzumab is well tolerated, has a pharmacokinetic profile which supports 3-week dosing, and is clinically active, suggesting that inhibition of dimerization may be an effective anticancer strategy.

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INTRODUCTION

The role of HER-1 (epidermal growth factor receptor [EGFR]), HER-2, and HER-3 in many epithelial tumor types is well documented in breast cancer,[1,2] lung cancer,[3] prostate cancer,[4,5] colorectal cancer,[6] and ovarian cancer.[7] There is a strong association between the human epidermal growth factor receptor (HER/ErbB), tyrosine kinases (HER-1/EGFR, HER-2/ErbB2, HER-3/ErbB3, and HER-4/ErbB4), and the aggressiveness and prognosis of several types of solid tumors.[8-10]

Dimerization, or pairing with other receptor proteins, is essential for HER receptor activity and may have a major role in driving growth and survival in many tumor types.[11-13] Following binding of a specific

ligand, HER receptors may form homodimers (eg, HER-1-HER-1) or heterodimers (eg, HER-1-HER-2 or HER-2-HER-3). Ligand-activated HER dimers then initiate a series of intracellular signaling events that ultimately mediate cell growth, proliferation, and survival.[14,15] Characterization of the crystal structure of the extracellular domain of HER-2 demonstrated that the protein adopts a constitutively open conformation similar to that of ligand-bound EGFR (HER-1).[16-19] This conformation means that HER-2 is always activated and ready to interact with other ligand-bound HER receptors, which may explain why it is the preferred binding partner for the other three family members.[12,16]

In vitro and in vivo experiments have demonstrated that HER-2-containing heterodimers elicit greater mitogenic responses than HER homodimers. Thus, ligand-induced activation of either EGFR (HER-1) or HER-3, with subsequent formation of heterodimers with HER-2, may play an important role in tumor growth[6,20-23] and resistance to therapy.[24]

Pertuzumab (recombinant humanized monoclonal antibody 2C4; Omnitarg; Genentech Inc, South San Francisco, CA) represents the first in a new class of agents known as HER dimerization inhibitors. Pertuzumab is based on the human immunoglobulin (Ig) G1 (κ) framework sequences, consisting of two heavy chains (449 residues) and two light chains (214 residues), and is produced in Chinese hamster ovary cells. Pertuzumab binds to HER-2, the most common HER pairing partner, at the dimerization domain,[25] sterically inhibiting its ability to form dimers with other HER receptors.[23,26-30] The pertuzumab binding site within domain II does not overlap with the epitope on HER-2 that is recognized by trastuzumab (Herceptin; Genentech Inc, South San Francisco, CA).[17,31] The mechanism of action of pertuzumab is also distinct from tyrosine kinase inhibitors such as gefitinib or erlotinib, which bind competitively to the intracellular adenosine triphosphate binding site of HER receptors.[8]

In vitro studies with tumor cell lines have shown that pertuzumab inhibits ligand-activated HER dimerization.[23,27] Pertuzumab was more effective than trastuzumab in disrupting the formation of HER-1-HER-2 and HER-3-HER-2 complexes in breast and prostate cancer cell lines.[23] Blocking the formation of HER-2-containing dimers in a number of different tumor types diminished ligand-activated HER signaling, including HER-2 phosphorylation and activation of downstream targets such as mitogen-activated protein kinase and Akt.[23,32] Both intact pertuzumab and the Fab fragment blocked heregulin-stimulated phosphorylation of the HER-2-HER-3 complex, indicating that bivalency is not an absolute requirement for activity of the antibody.[23]

The biologic activity of pertuzumab has been shown in preclinical models involving a number of tumor cell types, including breast, prostate, lung, ovarian, and colon cancers.[23,27,30,32-36] In dose-response studies, dosing of pertuzumab at doses of 6, 20, and 60 mg/kg weekly was associated with significant and dose-dependent inhibition of tumor growth.[23] Steady-state trough concentrations of pertuzumab at these dose levels were 5 to 25 μg/mL.[34,36] Inhibition of growth was independent of HER-2 expression levels, which means that pertuzumab had activity in cancer cells with either low/normal levels of HER-2 expression and cells overexpressing HER-2. In contrast, artificially engineered cells that completely lack the *HER-2* gene (ie, HER-2-null cells) do not respond to pertuzumab[35]; of note, most human epithelial cells, including malignant cells, do express HER-2.[9]

Pharmacokinetic studies of pertuzumab evaluated doses of 3 to 90 mg/kg in mice and rats, and 15 to 150 mg/kg in cynomolgus monkeys. Concentration-time data exhibited biphasic disposition and were fit to a two-compartmental pharmacokinetic model. At these doses, dose linearity was observed and pertuzumab pharmacokinetics were characterized by a distribution phase of < 1 day, a terminal elimination half-life of approximately 10 days, and a volume of distribution of 30 to 50 mL/kg, which approximates to the serum volume. Pertuzumab disposition in mice and cynomolgus monkeys was similar to that observed with other humanized monoclonal antibodies (eg, trastuzumab, bevacizumab, and omalizumab) that share the same IgG1 framework.

Diarrhea was the only significant finding in standard toxicology studies conducted in rodent and primate models. No cardiotoxicity was observed in primate models.[37]

In light of these promising nonclinical findings, it is postulated that inhibition of HER-2-mediated dimerization may be an effective anticancer therapy. This study is the first report of administration of pertuzumab to cancer patients in a phase I clinical trial, including patients with tumors that did not show amplification of HER-2.

The specific objectives of the study were to determine the safety and tolerability of escalating doses of pertuzumab administered by intravenous infusion every 3 weeks in subjects with incurable, locally advanced, recurrent or metastatic solid malignancies; to characterize the pharmacokinetics of pertuzumab; and to define an optimal dose and schedule for phase II testing. In addition, a preliminary assessment of clinical response to pertuzumab was made.

Patients and Methods

Patient Population

The study population consisted of patients with incurable, locally advanced, recurrent or metastatic solid tumors that had progressed during or after standard therapy. All patients gave written informed consent and the protocol was reviewed and approved by institutional review boards at participating institutions. Patients enrolled onto the study were at least 18 years

old, with an Eastern Cooperative Group performance status of 0 or 1, and a life expectancy of at least 12 weeks. Other inclusion criteria were: histologic documentation of malignancy; at least one bidimensionally measurable lesion (except for patients with prostate cancer and ovarian cancer in whom rises in prostate-specific antigen [PSA] and CA-125, respectively, were allowed); HER-2-negative status in patients with breast cancer; and adequate hematology, liver function, and biochemistry tests (neutrophils $\geq 1.5 \times 10^9$/L, platelets $\geq 100 \times 10^9$/L, hemoglobin ≥ 9 g/dL; serum bilirubin $\leq$ upper limit of normal [ULN] and alkaline phosphatase, AST and ALT $\leq 2.5 \times$ ULN, except in patients with liver or bone metastases [ALT, AST, and alkaline phosphatase $\leq 5 \times$ ULN]; serum creatinine $\leq$ ULN, or creatinine clearance ≥ 60 mL/min; international normalized ratio <1.3 and activated partial thromboplastin time $< 1.5 \times$ ULN). Women of childbearing age had to use an effective means of contraception. Patients with pleural effusions, ascites, or bone lesions as the only sign of malignancy, and those with symptomatic or untreated brain metastases, were excluded. Patients who had received any of the following prior treatments within 4 weeks of the study were excluded: chemotherapy (within 6 weeks for nitrosoureas or mitomycin C), hormone therapy (except for androgen-deprivation therapy in patients with prostate cancer), radiotherapy, and immunotherapy. Patients who had previously received trastuzumab or a cumulative doxorubicin dose > 360 mg/m^2 were also excluded. Patients were also excluded if they had a history of other malignancies within 5 years, history of significant cardiac disease, left ventricular ejection fraction (LVEF) $< 50\%$ (or below the lower limit of normal determined by echocardiography), active infection requiring intravenous antibiotics, known HIV infection, uncontrolled hypercalcemia, pregnancy, and liver disease, or any other condition likely to increase the risk of treatment complications.

Treatment and Dose Escalation Schedule

Pertuzumab was provided by Genentech Inc. Each 10 cc single-use vial contained approximately 175 mg pertuzumab formulated in 10 mmol/L L-histidine (pH6.0), 240 mmol/L sucrose, and 0.02% polysorbate 20. Patients were assigned to receive pertuzumab at one of five sequentially increasing dose levels based on a modified Fibonacci design: 0.5, 2.0, 5.0, 10.0, and 15.0 mg/kg, given every 3 weeks. As pertuzumab caused minimal toxicity in preclinical experiments, the starting dose of 0.5 mg/kg was based on an estimate of the dose required to achieve a plasma concentration of pertuzumab that would fall below that required for saturation of clearance. The upper dose of 15.0 mg/kg was based on an estimate of the dose required to exceed serum trough concentrations of pertuzumab that were associated with efficacy in preclinical models (25 μg/mL). Based on pharmacokinetic simulations, doses from 5.0 to 15.0 mg/kg administered every 3 weeks were expected to achieve these targeted serum trough concentrations in most subjects. The initial dose of 0.5 mg/kg represented a safety factor (on a mg/kg basis) of 300-fold less than the highest dose (150 mg/kg) used in the multidose toxicology studies in cynomolgus monkeys. Doses were escalated to 15.0 mg/kg, representing a safety-factor of 10-fold. The first dose of pertuzumab was given by intravenous infusion over 90 minutes. If the initial infusion was well tolerated, the infusion time was reduced to 30 minutes for subsequent infusions. At least three patients were treated at each dose level and monitored for at least 3 weeks plus 24 hours after their second cycle before additional patients could be started at the next dose level. If patients withdrew for reasons other than toxicity after completing only one cycle, they were to be replaced at the same dose level. If any patient experienced dose-limiting toxicity (DLT), three more patients were to be added at the same dose level. In this situation, dose escalation would continue only if no more than one in six patients experienced DLTs in cycle one. Intolerable dosage was defined as two or more patients experiencing DLT (any grade 3 or 4 major organ toxicity according to the National Cancer Institute Common Toxicity Criteria [NCI-CTC] Version 2.0, or any subjectively intolerable toxicity felt by the investigator to be related to study drug).

Tumor Assessments

The response evaluation criteria in solid tumors (RECIST) were used to assess objective response, time to disease progression, and duration of response.[38] Tumor burden was evaluated at baseline by physical examination and imaging, including computed tomography of chest, abdomen, and pelvis, and bone scans when clinically indicated. Responses were assessed by identical techniques at the end (week 3) of cycles 2 and 4, and every two cycles subsequently. Objective responses were confirmed by repeat assessments after ≥ 4 weeks.

Tolerability and Safety

The incidence and severity of all adverse events, changes in vital signs, laboratory assessments, physical examination findings, and medical conditions during and after treatment with pertuzumab were assessed at least weekly during the first two cycles, and at least 3-weekly thereafter, and graded according to NCI-CTC Version 2. Laboratory evaluations included full blood count with differential, electrolytes, liver and renal function, international normalized ratio and partial thromboplastin time, urinalysis, and troponin T. Special attention was paid to the possibility of infusion-associated symptoms and allergic reactions, cardiotoxicity, as reported with trastuzumab and anthracyclines, and toxicities, such as diarrhea and rash, previously observed in association with administration of EGFR tyrosine kinase inhibitors.

Cardiac function (reported as LVEF) was monitored at baseline and every 6 weeks using noninvasive cardiac monitoring (two-dimensional echocardiography and Doppler). In addition, electrocardiographs were obtained at screening and follow-up, and serum markers of cardiac damage (troponin T) were collected periodically for exploratory analyses.

Analysis of Pharmacokinetics

Pharmacokinetic parameters were estimated from pertuzumab concentrations measured by a central laboratory using serum samples taken at specified time points postdosing. In treatment cycle 1, samples were taken predose, immediately before completion of the first 90-minute infusion, at 1.5, 4, and 8 hours after the completion of the first infusion (day 1), and on days 2, 5, 8, and 15. For treatment cycle 2 and beyond, samples were taken predose, immediately before the completion of the 30-minute infusion, and on day 8. Concentrations of pertuzumab were determined by a receptor-binding, enzyme-linked, immunosorbent assay (ELISA). The assay used p185^{HER-2} extracellular domain to capture pertuzumab from serum samples. Bound pertuzumab was detected with mouse antihuman Fc-horseradish peroxidase (Jackson ImmunoResearch Laboratories Inc, West Grove, PA), and tetramethyl benzidine (KPL Inc, Gaithersburg, MD) was used as the

substrate for color development to quantify serum pertuzumab against a known standard curve. The assay can detect a minimum quantifiable concentration of 0.25 μg/mL for pertuzumab in human serum. The assay has < 20% coefficient of variation for interassay and intra-assay variability, and serum spike recoveries of pertuzumab between 80% and 120%.

Antibody Response

Serum samples, taken at predose and at the last study visit, were tested for antipertuzumab antibody titers using a bridging ELISA. The assay used pertuzumab to capture antipertuzumab antibodies, and then the presence of bound antipertuzumab antibodies were detected with biotinylated pertuzumab and streptavidin horseradish peroxidase, using tetramethyl benzidine as a substrate for color development. An antibody titer of > 2.0 was considered positive.

Data Analysis

Demographic and baseline characteristics were recorded as means (± standard deviation) or medians (with ranges) for continuous variables and proportions for categoric variables. Means (± standard deviation) were used to summarize average dose of pertuzumab received. All adverse events occurring on or after day 1 were recorded and analyzed by dose group, study site, subject number, and treatment cycle. Serious adverse events and DLTs experienced in the first two cycles and overall were listed separately. Mean serum pertuzumab concentrations versus nominal sampling times were plotted by dose group for the first two treatment cycles. Serum pharmacokinetic parameters were estimated for individual subjects using compartmental methods (WinNonlin; Pharsight Corp, Mountain View, CA) and summarized by dose group (mean, standard deviation) for systemic clearance, volume of distribution of the central compartment, volume of distribution at steady-state, and elimination half-life. A one-compartment model was used to fit the pharmacokinetic data from the 0.5 mg/kg dose group and a two-compartment model was used to fit the pharmacokinetic data from the 2.0 to 15.0 mg/kg dose groups. The algorithm and weighting scheme used in the WinNonlin models were a Gauss-Newton algorithm and a reiterative weighting scheme ($1/\hat{Y}$).

RESULTS

Patients

A total of 21 patients (12 female, nine male), with a median age of 61 years (range, 32 to 77 years), were enrolled between November 2001 and June 2003. There were three patients with breast cancer, five with prostate cancer, four with non–small-cell lung cancer, three with ovarian cancer, two with colorectal cancer, two with pancreatic cancer (one adenocarcinoma and one islet cell carcinoma), one with liposarcoma, and one with adenocarcinoma of unknown primary. Patient demographics, baseline characteristics, and prior treatments are listed in Table 1. All patients were heavily pretreated: 90% had received prior chemotherapy (median of two regimens; range, 0 to 5), 48% prior radiotherapy, 67% prior surgery, and 24% prior hormone therapy.

Table 1. Patient Characteristics

	Total (N = 21)	
	No. of Patients	%
Age, years		
Median	61	
Range	32–77	
Sex		
Male	12	
Female	9	
ECOG performance status		
0	6	
1	15	
Location of primary tumor		
Breast	3	
Colorectal	2	
Lung	4	
Ovarian	3	
Pancreas	2	
Prostate	5	
Sarcoma	1	
Adenocarcinoma of unknown origin	1	
Primary diagnosis		
≤ 1 year	5	
≤ 2 years	6	
≥ 3 years	9	
Metastatic diagnosis		
≤ 1 year	5	
≤ 2 years	8	
≥ 3 years	8	
Prior chemotherapy	19*	90
Prior radiotherapy	10	48
Surgery	14	67
Hormonal therapy	5	24
Other prior therapy	6	29

Abbreviations; ECOG, Eastern Cooperative Oncology Group.
*Two patients with prostate cancer had received no prior chemotherapy.

Pertuzumab was given to at least three patients at all the planned dose levels: three patients received 0.5 mg/kg, three received 2.0 mg/kg, four received 5.0 mg/kg, three received 10.0 mg/kg, and eight received 15.0 mg/kg. All patients, except one in the 5.0 mg/kg dose group and one in the 15.0 mg/kg group, were assessable for DLTs. Nineteen patients completed a minimum of two cycles of pertuzumab and 10 of these patients were treated beyond cycle 2.

Safety and Tolerability

Pertuzumab was generally well tolerated at all dose levels and a maximum tolerated dose was not reached with this dose escalation scheme. There were 365 adverse events reported, irrespective of relationship to study drug, and those occurring in > 20% of patients are shown in Table 2. The most common adverse events were asthenia (62%), vomiting (52%), nausea (48%), abdominal pain (48%), rash (43%), diarrhea (43%), pain (43%), and anemia (33%), most of which were either grade 1 or grade 2 in severity. Twelve of 21 subjects experienced at least one grade 3 or 4 adverse event, although only six events were thought to be related to study medication. Four of these events occurred in one subject and were associated with a myocardial infarction (see description in next paragraph). There was no clear pattern of temporal relationship between onset of

Table 2. Adverse Events Irrespective of Relationship to Study Drug Occurring in > 20% of Patients

Adverse Event	Total (N = 21)		Pertuzumab Dose Group (mg/kg)					Cycle Event First Observed					
								Cycle 1 (n = 21)		≥ Cycle 2 (n = 19)		Grade 3 or 4 (n = 21)	
	No. of Patients	%	0.5 (n = 3)	2.0 (n = 3)	5.0 (n = 4)	10.0 (n = 3)	15.0 (n = 8)	No. of Patients	%	No. of Patients	%	No. of Patients	%
Asthenia	13	62	3	1	3	1	5	5	24	9	47	0	0
Vomiting	11	52	3	1	4	0	3	5	24	6	32	1	5
Nausea	10	48	3	1	2	2	2	7	33	3	16	1	5
Abdominal pain	10	48	2	1	2	1	4	3	14	7	37	3	14
Rash	9	43	0	2	2	2	3	5	24	4	21	0	0
Diarrhea	9	43	0	2	2	1	4	5	24	6	32	1	5
Pain	9	43	0	1	2	1	5	6	29	3	16	0	0
Anemia	7	33	2	0	0	2	3	3	14	4	21	0	0
Dyspepsia	6	29	1	0	1	1	3	1	5	5	26	0	0
Fever	6	29	2	1	1	0	2	1	5	5	26	0	0
Anorexia	6	29	1	0	3	0	2	2	10	4	21	0	0
Infection	5	24	1	1	1	1	1	1	5	4	21	0	0
Constipation	5	24	2	0	1	0	2	2	10	3	16	0	0
AP increase	5	24	1	1	0	1	2	1	5	4	21	1	5
Dyspnea	5	24	2	0	0	1	2	3	14	2	10	2	10
Any event	21		3	3	4	3	8	19	90	18	95	12	57

Abbreviation: AP, alkaline phosphatase.

events and administration of study medication, and no evidence of cumulative toxicity in those few patients who remained on therapy beyond cycle 2.

Specifically, rash and diarrhea were observed, but were mainly grade 1 and occasionally grade 2. Again, no obvious temporal relationship to infusion of study drug was observed, nor could a relationship between incidence and/or severity and dose be found. Rash was not acneform and did not resemble the rash typical of EGFR kinase inhibitor therapy. None of the patients experienced a decline in LVEF below 50% in first two cycles. Three patients had an asymptomatic fall in LVEF of 5 percentage points or more at any time during the study: one patient had a fall in LVEF from 65% to 60%, one from 60% to 50%, and one from 64% to 50%. After cycle 2, one patient suffered a myocardial infarction resulting in left ventricular failure (grade 4). This event was considered possibly related to treatment. The patient had a previous history of cerebrovascular disease, hypertension, type 2 diabetes, mitral tricuspid and aortic regurgitation, and hyperglycemia, and was taking atenolol, clopidogrel, and enalapril at study entry. Baseline LVEF was 62% and echocardiogram revealed mild left atrial enlargement and a borderline concentric left ventricular hypertrophy. Multiple echoes in the aortic root were suggestive of calcification. No other evidence of cardiac dysfunction, including rise in troponin T, was observed in any other patient.

One patient experienced a grade 3 gastrointestinal hemorrhage that was considered possibly related to treatment. The patient had pre-existing esophageal varices that may have contributed to the adverse event. The event was recorded as a DLT.

One patient developed an infusion-related reaction to pertuzumab (0.5 mg/kg) during the fifth infusion. The reaction was defined as grade 1 fever and chills experienced during the infusion, and was not associated with dyspnea or other respiratory problems. The patient discontinued pertuzumab treatment after this cycle because of symptomatic progression of underlying tumor. No other infusion-related problems were reported.

There were no clear relationships between any specific toxicity and dose level, and no detectable differences in the incidence or severity of adverse events between the first cycle and subsequent cycles.

No antibodies to pertuzumab were detected.

Serum Pharmacokinetics

Mean pertuzumab concentration versus time curves during cycles 1 and 2 for each dose level are shown in Figure 1. At pertuzumab doses of 5.0 to 15.0 mg/kg, mean

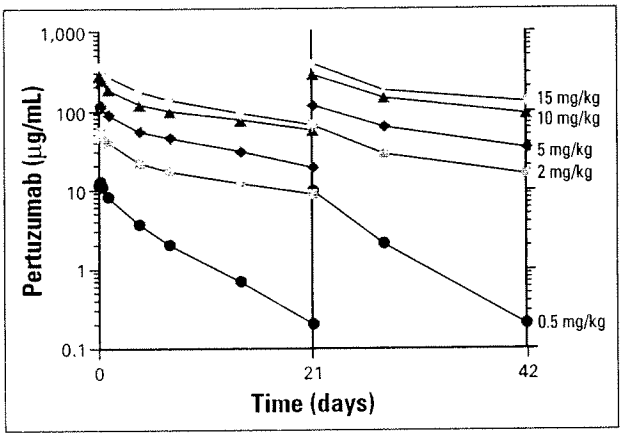

Fig 1. Pertuzumab concentration versus time curves during first two cycles of treatment.

serum concentrations were maintained above 20 μg/mL for the first two treatment cycles. Pertuzumab cleared faster at the 0.5 mg/kg dose compared with the 2.0 to 15.0 mg/kg doses where serum concentrations declined rapidly over the first 2 to 3 days and then more slowly thereafter.

Pertuzumab pharmacokinetic parameters estimated by compartmental modeling are shown in Table 3. At doses of 2.0 to 15.0 mg/kg, systemic clearance, volume of distribution of the central compartment, volume of distribution at steady-state, and the elimination half-life did not change with dose. At these doses, the mean systemic clearance ranged from 2.69 to 3.74 mL/d/kg (grand mean, 3.42 ± 1.20 mL/d/kg), the volume of distribution of the central compartment approximated the serum volume, with means ranging from 35.5 to 42.8 mL/kg (grand mean, 40.6 ± 6.6 mL/kg), and the mean volume of distribution at steady-state ranged from 69.5 to 85.3 mL/kg (grand mean, 80.0 ± 28.2 mL/kg). Consistent with the faster clearance at the 0.5 mg/kg dose, a shorter mean elimination half-life of 2.6 ± 0.9 days was observed compared with the 2.0 to 15.0 mg/kg doses that had longer elimination half-lives with means ranging from 14.9 to 22.3 days (grand mean, 18.9 ± 8.0 days).

Antitumor Activity

Twenty of the 21 patients treated in the study had measurable disease by RECIST at entry to the study and had tumor response assessed. Partial responses (PRs) were obtained in two patients: one patient with ovarian cancer and one with islet cell carcinoma of the pancreas (Table 4).

The patient with ovarian adenocarcinoma had intra-abdominal disease that, at the time of study entry, was progressing within 6 months of previous platinum-based chemotherapy (platinum-resistant disease). CA-125 was normal at the time of study entry. This patient achieved a PR after 6 weeks of treatment with pertuzumab at 5.0 mg/kg (Fig 2). The response lasted for 11 months and the patient remained on therapy for 13 months before being withdrawn due to intercurrent illness unrelated to malignancy. Duration of progression-free survival was therefore not captured.

The patient with pancreatic islet cell cancer had tumor progression 55 days after completion of doxorubicin, fluorouracil, and streptozocin. He received 22 cycles of treatment with pertuzumab (15.0 mg/kg). PR was confirmed after 6 months of pertuzumab therapy, in association with improvement in symptoms and normalization of liver dysfunction (Fig 3) and duration of stable disease was 15.3 months.

Neither tumor from the two patients with PRs were found to overexpress HER-2. Amplification of the *HER-2* gene was not observed with fluorescence in situ hybridization in the responding patient with ovarian cancer, and HER-2 overexpression was not detected by immunohistochemistry in the responding patient with islet cell carcinoma.

One additional patient with prostate cancer had tumor regression but this could not be confirmed as a response at ≥ 4 weeks because the patient was withdrawn from the study shortly after initial documentation of tumor shrinkage due to toxicity, specifically a myocardial infarction. This patient had androgen-independent disease that was progressing despite initial treatment with docetaxel followed by five cycles of doxorubicin. Regression in the size of hepatic lesions and periaortic lymph nodes was observed 6 weeks after initiation of treatment with pertuzumab (15.0 mg/kg), with > 30% reduction after 3 months. PSA doubling time was approximately 2 months at study entry and stabilized upon treatment (366 at study entry, 384 after two cycles).

Stable disease of greater than 2.5 months duration (range, 2.6 to 5.5 months) was observed in six patients (29%). In these patients, the durations of stable disease were 2.6, 2.7, and 5.5 months in three patients with prostate cancer (treated with 0.5, 10.0, and 10.0 mg/kg pertuzumab, respectively), 4.1 months in a patient with lung cancer (2 mg/kg), 2.8 months in a patient with colorectal cancer (5 mg/kg), and 4.0 months in a patient with ovarian cancer

Table 3. Pertuzumab Pharmacokinetic Parameter Estimates Following Intravenous Infusion

	CL (mL/d/kg)		V_c (mL/kg)		V_{ss}* (mL/kg)		$t_{1/2}$ terminal (days)	
Dose Group (mg/kg)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.5 (n = 3)	13.1	5.5	43.6	4.6	NA	NA	2.6	0.9
2.0 (n = 3)	3.74	1.28	35.5	3.5	69.5	13.7	14.9	1.1
5.0 (n = 3)†	3.14	0.43	42.1	4.8	82.2	30.5	20.6	9.6
10.0 (n = 3)	2.69	0.92	38.4	5.3	73.4	13.6	22.3	9.7
15.0 (n = 8)	3.68	1.47	42.8	7.9	85.3	36.7	18.6	8.8
Grand mean (n = 17)‡	3.42	1.20	40.6	6.6	80.0	28.2	18.9	8.0

Abbreviation: CL, systemic clearance; V_c, volume of central compartment; V_{ss}, steady-state volume of distribution; $t_{1/2}$ terminal, elimination half-life; NA, not applicable.
*Available only for dose groups in which a two-compartment model was used.
†One subject not analyzed due to incomplete pharmacokinetic sampling.
‡Grand mean does not include the 0.5 mg/kg dose group.

Table 4. Characteristics of Patients Achieving Tumor Regression With Pertuzumab

Primary Diagnosis	Age (years)	No. of Previous Systemic Therapies	Previous Systemic Therapies	Dose of Pertuzumab (mg/kg)	Response to Pertuzumab	Duration of Response (months)	Time to Response (months)	Time on Therapy (months)
Pancreatic islet cell carcinoma	57	1	Doxorubicin	15	Partial	10	6	16
Prostate cancer	65	9	Docetaxel; doxorubicin; multiple hormonal therapies	15	Partial	0.5*	3	3
Ovarian cancer	42	1	Carboplatin, paclitaxel, gemcitabine	5	Partial	11+†	1.5	13

*Patient was removed from study due to toxicity while still responding.
†Patient withdrew from study after 13 months due to intercurrent illness unrelated to pertuzumab therapy.

(15.0 mg/kg). CA-125 was unaltered in the patient with ovarian cancer. One patient with prostate cancer and an initial PSA of 300 ng/mL had a transient fall, and another patient with prostate cancer and a low initial PSA (3.0 ng/mL) had a drop to 0.4 ng/mL that lasted several months.

DISCUSSION

Pertuzumab is a humanized monoclonal antibody that inhibits tumor growth and survival by a novel mechanism of action—inhibition of dimerization of HER-2 with other ligand-activated HER kinases.

Pertuzumab was generally well tolerated. A maximum tolerated dose of pertuzumab was not reached at doses up to 15 mg/kg. Because of experience with trastuzumab,[39] the potential for cardiotoxicity was closely monitored throughout the study, but no evidence of a clinically significant reduction in left ventricular function was observed, with the exception of one patient who had an intercurrent myocardial infarction. Continued monitoring for cardiac toxicity will be required in future studies. Therapies targeting EGFR have all been associated with acne-like rash, and orally administered receptor tyrosine kinase inhibitors also with diarrhea.[40-43] Although rash and diarrhea appear to be caused by inhibition of EGFR, the precise mechanisms for these effects are unknown.

Pharmacokinetic results indicated that pertuzumab disposition in humans is similar to that expected for an IgG1 monoclonal antibody, such as trastuzumab,[44] which has a serum clearance of 3 to 4 mL/d/kg and a terminal half-life of approximately 3 weeks. This similarity to trastuzumab was expected based on allometric interspecies scaling

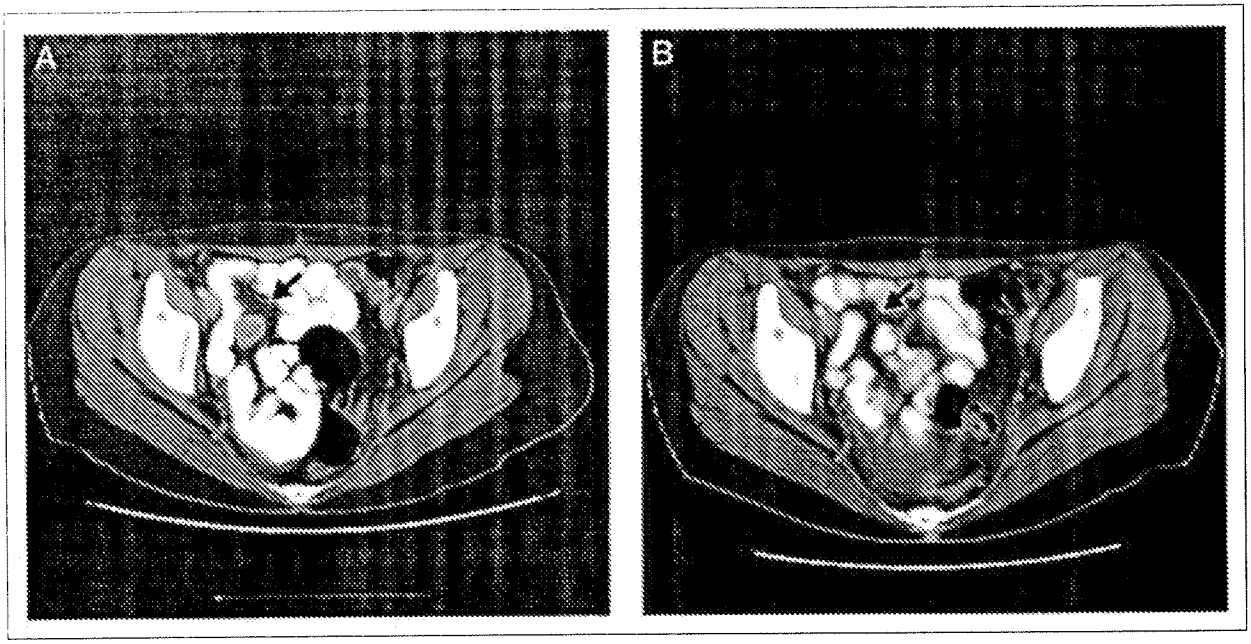

Fig 2. Computed tomography scan showing partial response (5.0 mg/kg) in 45-year-old patient with ovarian cancer previously treated with gemcitabine, paclitaxel, and carboplatin (six cycles with disease progression). (A) Before pertuzumab (January 2002): 7.17 cm measurement of longest diameter, three lesions involved, multiple nontarget lesions; (B) after pertuzumab (April 2003): 3.17 cm longest diameter, two lesions resolved, one unchanged, nontarget lesions resolved.

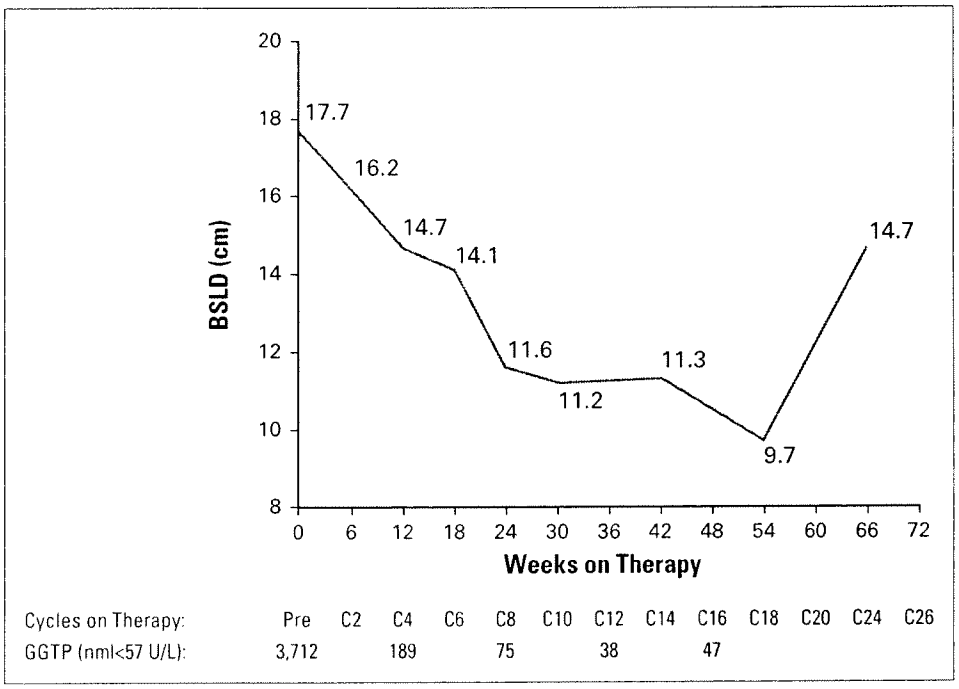

Fig 3. Kinetics of response to pertuzumab (15.0 mg/kg) in a 59-year-old patient with pancreatic islet cell carcinoma previously treated with fluorouracil, streptozocin, and doxorubicin (two cycles with disease progression). BSLD, baseline sum of longest diameters; GGTP, gamma glutaryl transferase; nml, normal range.

to humans and similarities in target antigen, IgG1 framework, and binding affinity. Pertuzumab also shares similar pharmacokinetics to bevacizumab[45] for the same reasons. The mean terminal half-life for the 2.0 to 15.0 mg/kg dose groups in this study ranged from 14.9 to 22.3 days. A terminal half-life of 2 to 4 weeks supports dosing pertuzumab at 3-weekly intervals in human clinical studies and, using this administration schedule, steady-state concentrations would be attained in approximately 90 days. In this study, pertuzumab infusions given every 3 weeks at doses greater than 5.0 mg/kg ensured that serum concentrations remained in excess of 20 μg/mL. Dose-response studies of pertuzumab in nonclinical models showed that $>$ 80% suppression of tumor growth is achieved at steady-state trough concentrations of approximately 5 to 25 μg/mL.[34] A dosing regimen for pertuzumab in phase II studies was, therefore, recommended on the basis of these dose-response data in nonclinical models and the trough concentrations and time to achieve steady-state concentrations after administration of pertuzumab to subjects in this phase I study. The recommended regimen consists of a fixed dose of 420 mg (equivalent to doses of 6 mg/kg for a 70 kg patient) every 3 weeks. A loading dose of 840 mg (12 mg/kg equivalent) will be administered to achieve steady-state concentrations rapidly. This regimen is expected to result in the rapid attainment of steady-state serum concentrations shown to be efficacious in animal tumor models and predicted to achieve a biologic effect in subjects.

The clinical activity observed in this study in two patients with advanced malignancy, both of whom were shown to have tumors that did not overexpress HER-2, corroborates preclinical studies and suggests that inhibition of HER dimerization may be a novel clinical approach to cancer treatment. Both responses were of meaningful duration and were observed in a diverse range of tumor types. All of the patients with tumor regressions had failed previous chemotherapy regimens.

Based on the preclinical data, we propose that the observed clinical activity of pertuzumab results from HER dimerization inhibition. However, the effect of pertuzumab on HER dimerization was not directly measured in this study; therefore, evidence of tumor regression and disease stabilization can be regarded as only supportive of the putative mechanism of action. In order to provide a molecular basis for understanding the mechanism of action of pertuzumab, a recent study determined the x-ray crystal structure of the soluble extracellular domain of HER-2 in a complex with the antigen-binding fragment of pertuzumab.[25] The study showed that pertuzumab binds within a cysteine-rich region of the extracellular domain of HER-2, domain II, a region of the HER receptor family that is highly conserved and previously shown to be necessary for HER-1 homodimerization.

Although antibody-dependent cellular cytotoxicity cannot be excluded as contributing to the clinical activity observed in this study, pertuzumab does not require antibody-dependent cellular cytotoxicity for efficacy because an intact Fc region is not required for activity.[23,46] In addition, pertuzumab does not share the inhibitory effect of trastuzumab on HER-2 cleavage.[47]

Interestingly, the presence of HER-2-containing heterodimers appears to predict responsiveness to

pertuzumab in vivo in non–small-cell lung cancer and breast cancer models.[48] In these models, HER-2/HER-3 heterodimers were detected in 100% of xenografts whose growth in nude mice was inhibited by pertuzumab compared with 17% that were not sensitive to pertuzumab. EGFR (HER-1), HER-2, and HER-3 protein expression levels were not predictive of responsiveness to pertuzumab and the phosphorylation level of HER-2 or either of its dimerization partners, which would indicate an activated state of these receptors, was found to be a less reliable marker for response prediction than the presence of heterodimers. Detection of heterodimers or activation of HER-2 may therefore be a possible approach to select patients for HER dimerization inhibitor therapy, and clinical studies are ongoing to address this question.

In conclusion, these preliminary data suggest that inhibition of HER dimerization may be a novel, potentially effective anticancer strategy. Furthermore, this study suggests that pertuzumab may have wider application than trastuzumab and may inhibit the growth of a number of tumor types. Additional studies are required to characterize the safety profile and optimal dosing regimen for pertuzumab, both as a single agent or combined with other anticancer strategies. Phase II studies are ongoing in patients with ovarian, prostate, non–small-cell lung cancer, and breast cancer. In addition, molecular characterization of patients' tumors should assist in identifying cancer patients who may respond to pertuzumab therapy.

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Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Owns stock (not including shares held through a public mutual fund): Stephen M. Kelsey, Genentech; Mark X. Sliwkowski, Genentech; Gwen Fyfe, Genentech; David E. Allison, Genentech; Gracie Lieberman, Genentech. Acted as a consultant within the last 2 years: David B. Agus, Genentech; Michael S. Gordon, Genentech; Ronald B. Natale, Genentech.

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